Paraoxonase (PON)1 Q192R functional genotypes and PON1 Q192R genotype by smoking interactions are risk factors for the metabolic syndrome, but not overweight or obesity

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Background: The metabolic syndrome (MetS) is a complex of multiple risk factors that contribute to the onset of cardiovascular disorder, including lowered levels of high-density lipoprotein (HDL) and abdominal obesity. Smoking, mood disorders, and oxidative stress are associated with the MetS. Paraoxonase (PON)1 is an antioxidant bound to HDL, that is under genetic control by functional polymorphisms in the PON1 Q192R coding sequence.

Aims and methods: This study aimed to delineate the associations of the MetS with plasma PON1 activity, PON1 Q192R genotypes, smoking, and mood disorders (major depression and bipolar disorder), while adjusting for HDL cholesterol, body mass index, age, gender, and sociodemographic data. We measured plasma PON1 activity and serum HDL cholesterol and determined PON1 Q192R genotypes through functional analysis in 335 subjects, consisting of 97 with and 238 without MetS. The severity of nicotine dependence was measured using the Fagerström Nicotine Dependence Scale.

Results: PON1 Q192R functional genotypes and PON1 Q192R genotypes by smoking interactions were associated with the MetS. The QQ and QR genotypes were protective against MetS while smoking increased metabolic risk in QQ carriers only. There were no significant associations between PON1 Q192R genotypes and smoking by genotype interactions and obesity or overweight, while body mass index significantly increased MetS risk. Smoking and especially severe nicotine dependence are significantly associated with the MetS although these effects were no longer significant after considering the effects of the smoking by PON1 Q192R genotype interaction. The MetS was not associated with mood disorders, major depression or bipolar disorder.

Discussion: PON1 Q192R genotypes and genotypes by smoking interactions are risk factors for the MetS that together with lowered HDL and increased body mass and age contribute to the MetS.

Keywords: Paraoxonase 1, Metabolic syndrome, Depression, HDL cholesterol, Oxidative stress, Cardiovascular

Introduction

The metabolic syndrome (MetS) is a combination of risk factors that are associated with an increased risk

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for cardiovascular disorder (CVD), cancer, type 2 diabetes mellitus, depression, and all-cause mortality.^{1–3} There are different case definitions for the MetS (e.g. the criteria of the International Diabetes Foundation, the National Cholesterol Education Program, and the World Health Organization), which all consider similar risk factors, including increased central obesity as measured by means of waist circumference, increased fasting glucose (insulin resistance) and triglyceride levels, lowered high-density lipoprotein (HDL) levels, and increased blood pressure.^{1,2,4,5} The MetS frequently coexists with obesity, as measured by an increased body mass index (BMI).⁶ In some studies, both the MetS and BMI contribute to increased CVD risk, whereas in other studies the MetS, but not BMI, predicts CVD risk.^{6,7}

The key components in the pathophysiology of the MetS syndrome are central obesity, insulin resistance and an increased flux of fatty acids stimulating elevated glucose production, and secretion of triglycerides and very low-density lipoproteins (LDLs).^{2,8} Lowered levels of HDL cholesterol and increased levels of LDL and leptin are accompanying key components of the MetS.⁴ Other theories, on the other hand, stress that central obesity is a compensatory mechanism and protects other non-adipose, lipid-sensitive tissues from the increased fatty acid spill over due to excess calories and dietary lipids in the presence of increased leptin resistance.⁹

New pathways that play a role in the MetS are activation of immune-inflammatory and oxidative and nitrosative stress (O&NS) pathways as indicated by increased levels of pro-inflammatory cytokines, acute phase proteins, and biomarkers of increased lipid peroxidation.¹⁰ In accordance with the findings on activated O&NS pathways, the MetS is also accompanied by lowered antioxidant defenses, as shown by reduced total antioxidant potential in the plasma.¹¹

Paraoxonase/arylesterase 1 (PON1, EC 3.1.8.1) is an antioxidant enzyme that is bound to plasma HDL and determines part of the antioxidant capacity of HDL.^{12,13} PON1 associated with HDL attenuates the production of reactive oxygen species (hydrogen peroxide) thereby contributing to lowered production of oxidized LDL.¹⁴ These antioxidant and anti-inflammatory effects of PON1 may explain that lowered levels are associated with an increased risk for CVD and insulin resistance.^{15–17} There is now evidence that the MetS is accompanied by lowered plasma PON1 activity or PON1 concentrations.^{18–23}

PON1 is under genetic control whereby functional polymorphisms at the 192 position Q→R determine the enzymatic activities and consequently increase risk to different diseases.²⁴ For example, the R allele and the RR genotype are associated with an increased risk for CVD, such as ischemic stroke.²⁴ The Q

isoform confers lowered protective activity against oxidation of LDL and HDL.²⁵ In a recent study, we found that PON1 Q192R genotypes were associated with an increased Castelli 1 index, suggesting increased atherogenic potential (de Souza-Nogueira *et al.*, submitted). There is one study showing that PON1 QR and RR genotypes significantly increase the risk of MetS.²⁶

Smoking is one of the environmental factors that may increase the risk of MetS. Thus, in a number of smaller studies an association between smoking and the MetS has been described.²⁷ A meta-analysis based on 13 studies and 56,691 subjects and 8,688 cases showed a significant positive relationship between smoking and the MetS.²⁸ Slagter *et al.*²⁹ in a large-scaled study showed that there was a positive association between smoking and the MetS, independent of BMI class and gender. Smoking not only affects the metabolism of fatty acids but also induces inflammatory reactions, increases lipid peroxidation, and lowers PON1 levels.^{30,31} In addition, we recently observed that an interaction pattern between smoking and PON1 genotypes predicts an increased Castelli 1 index and therefore could be an important risk factor for the MetS (submitted).

Another factor that is related to an increased risk towards MetS is the presence of both unipolar and bipolar depression.^{32,33} These mood disorders show a high degree of comorbidity with obesity, CVD, and diabetes type 2.³⁴ While increased rates of central obesity in mood disorders may be one factor explaining the comorbidity with the MetS, other shared processes are lowered HDL cholesterol and activated immune-inflammatory and O&NS pathways, and common environmental risk factors such as diet, smoking, and physical activity.^{34–40} Moreover, patients with mood disorders have an increased rate of current smoking⁴¹, while current smoking, interactions between PON1 Q192R genotypes and smoking, and reduced plasma PON1 levels are also risk factors for unipolar depression or bipolar disorder.⁴² Possible interactions between smoking, mood disorders, and PON1 Q192R genotypes could therefore play a role in the MetS.

The aim of the present study was to examine whether lowered PON1 activity and PON1 functional Q192R polymorphisms, e.g. the RR genotype, current smoking, and mood disorders or their interactions may increase the odds to MetS, while controlling for other putative intervening factors, such as BMI, age, gender, and sociodemographic data.

Subjects and methods

Subjects

In this study, we included 335 subjects of Caucasian, African, Asian, and mixed ethnicities and aged

between 18 and 60 years old. The subjects were recruited from staff at the Londrina State University and an outpatient ambulatory of smoking cessation program, UEL, Parana, Brazil, and comprised 238 subjects with the MetS and 97 subjects without the MetS; 146 subjects with mood disorders, that is unipolar major depression ($n = 91$) or bipolar disorder ($n = 45$), versus 199 subjects without mood disorders, and 144 smokers versus 191 non-smokers. We have excluded subjects (a) with abnormal laboratory tests, including aspartate transaminase, alanine transaminase, hemogram, urea, and creatinine; (b) who had been taken immunomodulatory drugs, including glucocorticoids and antiviral medications; and (c) with other major medical illness, e.g. diabetes type 1 and 2, chronic obstructive pulmonary disease, (auto)-immune disorders, inflammatory bowel disease, CVD, and neuroinflammatory disorders. Patients with mood disorders were excluded if they had a lifetime history of other axis I Diagnostic and Statistical Manual of Mental Disorders (DSM) IV diagnoses, such as organic mental disorders, schizophrenia, substance abuse disorders, anxiety disorders, etc., while subjects without mood disorders were excluded for any axis I DSM-IV diagnosis. The study was approved by the Institutional Review Board of the UEL (number 250/2010) and all subjects gave written informed consent prior to participating in this study.

Methods

The diagnosis of MetS was made using the diagnostic criteria of the International Diabetes Federation, i.e. three of the five criteria should be present: (a) abdominal obesity, that is waist circumference ≥ 90 cm for men and ≥ 80 cm for women in South Asian and South Americans and ≥ 94.0 cm for men and ≥ 80.0 cm for women in Caucasians; (b) low HDL cholesterol, that is < 40 mg/dl in men and < 50 mg/dl in women, or on hypolipidemic drugs; (c) hypertriglyceridemia, that is ≥ 150 mg/dl, or on a hypolipidemic agent; (d) increased fasting glucose, that is ≥ 100 mg/dl, or on oral antidiabetic medication; (e) increased average blood pressure, that is $\geq 130/85$ mmHg, or currently taking antihypertensive medication.^{2,5,43} We measured waist circumference during expiration, in a standing and relaxed position, at the midline between the lower costal margins and the iliac crest parallel to the floor. We measured blood pressure using a mercury sphygmomanometer on the right arm and used the mean value of two measurements that were carried out 5 minutes apart. We calculated the BMI as weight (in kg) divided by square of height (in m²).

The diagnosis of mood disorders was made by senior psychiatrists employing a validated Portuguese translation of the semi-structured interview of the

DSM-IV.⁴⁴ We have examined the effects of the two mood disorders combined and or of each mood disorder, either major depression or bipolar disorder separately. We combined patients in the acute phase of illness and patients in partial or total remission. We measured the severity of depression using a validated Portuguese translation of the Hamilton Depression Rating Scale (HAM-D), which was adapted for use in the Brazilian population.⁴⁵ The diagnosis 'nicotine dependence' was made according to DSM-IV criteria. The severity of dependence was estimated using the Fagerström Nicotine Dependence Scale.⁴⁶ We used the six items of this scale to examine possible relationships between aspects of nicotine dependence with the MetS, while we used the total score as an index for the severity of nicotine dependence. We also divided the subjects into these with severe nicotine dependence (total score ≥ 5) versus those with moderate dependence (total score < 4).⁴⁶ We used a self-report questionnaire to collect sociodemographic and clinical characteristics, including years of education, marital status (single, stable relationship, separated, or widowed), ethnicity (Caucasian, Asian, African, and mixed), use of alcohol (no use, monthly, weekly, daily use), and use of statins and any other medications.

Biomedical assays

Fasting blood (12–14 hours) was collected in all subjects for the assays of plasma PON1 activity, PON1 Q192R genotypes, and HDL cholesterol. PON1 status, that is PON1 plasma activity and PON1 Q192R functional genotypes or activity phenotypes, were measured as described before.⁴⁷ In short, the substrates were phenylacetate (PA, Sigma, St Louis, MO, USA) and 4-(chloromethyl)phenylacetate (CMPA, Sigma) and the analysis was conducted in a microplate spectrophotometer reader (EnSpire, Perkin Elmer, NY 14831, USA) using ultraviolet transparent 96-well microplates. All assays were carried out in triplicate and replicates that varied by $> 10\%$ were repeated. Briefly, CMPA hydrolysis was measured at 280 nm for 4 minutes at 25°C using 20 μ l of plasma diluted 1:40 in dilution buffer (20 mmol/l Tris-HCl (pH 8.0), 1.0 mmol/l CaCl₂). PA hydrolysis under high salt conditions was measured at 270 nm for 4 minutes at 25°C using 20 μ l of plasma diluted 1:40 in dilution buffer. High salt media was composed by PA added to 2 mol/l NaCl, 20 mmol/l Tris-HCl (pH 8.0), 1.0 mmol/l CaCl₂. The results obtained with these two assays were used to plot a two-dimensional enzyme activity graphic that displays rates of arylesterase activity (PA hydrolysis) under high salt conditions versus CMPase activity (CMPA hydrolysis). Since the polymorphism Q192R confers differential catalytic activity

against these two substrates, the plot splits the population into the three functional position 192 genotypes (QQ, QR, and RR). Measurement of PA hydrolysis at low salt concentrations reveals plasma PON1 total activity, since at this condition PON1 Q192R polymorphism does not influence PON1 catalytic activity against PA.⁴⁷ For this assay, rates of hydrolysis of PA under low salt conditions were measured at 270 nm for 4 minutes at 25°C using 20 µl of plasma diluted 1:80 in dilution buffer.

Fig. 1 shows the division of the participants into the three functional PON1 Q192R polymorphisms: 151 individuals (45.1%) were homozygous for the PON1*192Q allele; 133 (39.7%) were heterozygous; and 51 (15.2%) were homozygous for the PON1*192R allele. Regarding PON1 plasmatic activity, which is determined by the hydrolysis of PA under low salt conditions, it varied from 47.67 to 414.60 U/ml (data not shown).

HDL cholesterol was assayed using an automated method in a clinical chemistry system, Dimension[®] RXL (Siemens Healthcare Diagnostics Inc., Newark, DE, USA). This assay measures HDL cholesterol without sample pretreatment or specialized centrifugation steps. HDL cholesterol was used as a surrogate marker of HDL. Triglycerides and glucose were determined using automated methods in a clinical chemistry system, Dimension[®] RXL (Siemens Healthcare Diagnostics Inc.). The inter-assay coefficients of variability for all analytes were <5.0%.

Statistics

Analysis of variance (ANOVA) was used to check the differences in continuous variables between different categories. The Mann–Whitney *U* test was employed to examine intergroup differences in the case variables

Figure 1 Functional genotyping for the PON1 Q192R polymorphism through the hydrolysis of CMPA versus PA under high salt condition. Each data point indicates one individual.

that were not normally distributed. Analyses of contingency tables were checked using χ^2 tests to assess differences in the distribution of variables among study groups. We used Pearson's correlation coefficients to assess the relationships between variables. Binary logistic regression analyses were employed to delineate the associations between a dichotomous dependent variable (the presence or absence of a characteristic), i.e. the diagnosis MetS versus no MetS, and a set of independent variables, e.g. PON1 status and smoking and their interactions, and clinical/sociodemographic data. The logistic regression coefficients are employed to estimate odds ratios (*B* with 95% upper and lower confidence intervals for each explanatory variable in the final model). Multinomial regression analysis was used to investigate the associations between a dependent variable with more than two categories, i.e. three groups divided according to the BMI, and a set of independent variables, e.g. PON1 status and smoking and their interactions, and clinical/sociodemographic data. We used transformations to normalize the distribution of PON1 activity and years of education (ln transformation). We analyzed the data using the SPSS Versions 15 and 19. Statistical significance was set at $\alpha = 0.05$ (two-tailed).

Results

Demographic data

Table 1 displays the demographic data of the 335 subjects subdivided into those with and without MetS. We did not use a *P*-correction to examine the multiple contrasts as these univariate analyses were used a priori to delineate the possible relevant variables to be consecutively employed as determinants of independent association with the MetS or obesity/overweight in ultimate multivariate analyses. There were no significant differences in age and gender between both groups. There was a significant association between smoking and MetS with more smokers in the MetS group. Likewise, there was a significant association between MetS and groups divided according to non-smoking and moderate and severe nicotine dependence. There was no significant association between PON1 Q192R genotypes and MetS. There were similarly no significant differences in PON1 activity between subjects with and without the MetS. Subjects with a MetS had significantly lower levels of HDL cholesterol and higher levels of triglycerides and glucose, waist circumference, systolic blood pressure, and BMI than those without MetS. Likewise, there was a significant association between MetS and groups divided based on BMI values. There were no associations between MetS and mood disorders, unipolar depression and bipolar disorder, HAM-D, education, ethnicity, marital status, and use of statins or

Table 1 Demographic data of the 355 subjects in the study divided into those with ($n = 97$) and without ($n = 238$) the MetS

Variables	No MetS ($n = 238$)	MetS ($n = 97$)	F or χ^2 *	df	P
Age (years)	45.8 (8.1)	47.6 (8.9)	3.08	1/333	0.080
Gender (male/female)	78/160	38/59	1.25	1	0.264
Current smoking (no/yes)	146/92	45/52	6.29	1	0.012
Nicotine dependence: no/moderate/significant	146/27/65	45/15/37	6.29	2	0.043
Fagerström Nicotine Dependence Scale in smokers	5.53 (2.09) $n = 92$	5.92 (2.47) $n = 52$	1.02	1/142	0.315
QQ/QR/RR genotypes	115/92/v31	36/41/20	4.76	2	0.093
PON1 activity (U/ml)	195.5 (55.3)	185.2 (51.9)	2.46	1/333	0.117
HDL-cholesterol (mg/dl)	51.1 (14.6) $n = 234$	38.9 (9.6) $n = 97$	57.42	1/329	<0.001
Triglycerides (mg/dl)	105.5 (55.4)	192.9 (97.1)	107.42	1/333	<0.001
Glucose (mg/dl)	86.3 (8.1)	100.3 (25.5)	57.60	1/330	<0.001
Waist circumference (cm)	87.3 (13.2)	98.0 (11.3)	45.35	1/330	<0.001
Systolic blood pressure (mmHg)	117.2 (16.4)	134.2 (18.7)	66.8	1/321	<0.001
BMI (kg/m^2)	25.8 (4.4) $n = 236$	29.6 (5.0) $n = 96$	47.69	1/330	<0.001
BMI < 25, 25–30, $\geq 30 \text{ kg}/\text{m}^2$	117/88/31	12/47/37	47.93	2	<0.001
No mood – versus mood disorders	145/93	54/43	0.79	1	0.374
No mood – UP – BP	145/66/27	54/25/18	3.09	2	0.214
HAM-D	5.8 (7.9)	6.3 (7.4)	0.21	1/332	0.648
Education < 12 versus ≥ 12 years	94/144	51/46	4.80	1	0.028
Ethnicity C/A + A/M	168/34/36	61/16/20	2.07	2	0.356
Marital St/single/S + W	162/31/45	60/19/18	2.39	2	0.302
Statin use (yes/no)	18/207	8/83	0.054	1	0.817
Use of alcohol (no, 1, 2, 3, 4)	105/39/27/60/6	49/14/9/21/4	2.00	4	0.735
Use of any medication (yes/no)	119/117	66/31	8.64	1	0.003

Data are shown as mean ($\pm$ SD).

*Results of ANOVA or contingency tables with χ^2 test.

No mood – UP – BP, UP, unipolar depression; BP, bipolar disorder; HAM-D, Hamilton Depression Rating Scale; Ethnicity: C, Caucasian; A + A, Asian and African; M, mixed; Marital: St, stable relationship or married; S + W, separated and widowed; Use of alcohol (alcohol abuse is excluded): no, 1, sporadically; 2, monthly; 3, weekly; 4, daily.

alcohol. The use of any medication was significantly greater in subjects without MetS than in those with MetS.

The MetS and the Fagerström Nicotine Dependence Scale in smokers

Table 1 shows that in smokers there was no significant association between the MetS and moderate versus heavy nicotine dependence ($\chi^2 = 0.00$, $df = 1$, $P = 0.949$). Table 1 also shows that in smokers the Fagerström Nicotine Dependence Scale score did not differ significantly between those with and without the MetS. There was a significant relationship between MetS and the fourth item of the Fagerstrom scale, i.e. ‘how many cigarettes per day do you smoke?’, i.e. 10 or less (8/11), 11–20 (16/49), 21–30 (11/20), and 31 or more (17/12) ($\chi^2 = 10.39$, $df = 3$, $P = 0.016$). There were no significant associations between MetS and any of the other items of the Fagerström Nicotine Dependence Scale.

The MetS, smoking, and PON1 functional genotypes

Table 2 shows the stratification of the study population on the basis of PON1 Q192R genotypes and smoking while indicating for each subgroup the prevalence of MetS. The prevalence of MetS was significantly different in subgroups formed on the basis of smoking and QQ genotype but not QR and RR genotypes. In non-smokers, there were significantly more individuals with a MetS in QR carriers ($\chi^2 = 4.89$, $df = 1$, $P = 0.027$) and especially RR carriers ($\chi^2 = 11.3$, $df = 1$, $P < 0.001$) than in QQ carriers. In smokers, no such differences were observed.

Table 3 shows the results of two bivariate logistic regression analyses with MetS versus no MetS as a dichotomous dependent variable. Entering current smoking as the only explanatory variable showed that smoking was significantly associated with the MetS (Wald = 6.21, $df = 1$, $P = 0.013$). Entering moderate and severe nicotine dependence versus non-

Table 2 Stratification of the study population on the basis of PON1 Q192R genotypes and smoking and indicating for each subgroup the (relative) prevalence of the MetS

PON1 Q192R	Smoking	No MetS	MetS	χ^2	df	P
QQ	No	82 (84.5%)	15 (15.5%)	10.48	1	0.001
	Yes	33 (61.1%)	21 (38.9%)			
QR	No	52 (72.2%)	20 (27.8%)	0.68	1	0.408
	Yes	40 (65.6%)	21 (34.4%)			
RR	No	12 (54.5%)	10 (45.5%)	0.63	1	0.427
	Yes	19 (65.5%)	10 (34.5%)			

Table 3 Results of bivariate logistic regression analyses with MetS versus no MetS as dependent variable and the listed variables as explanatory variables

Variables	Wald	df	P	Odds ratio	95% Lower CI	95% Upper CI
Smoking	0.63	1	0.428	0.632	0.203	1.967
PON1 Q192R genotype	9.43	2	0.009	–	–	–
QQ	8.77	1	0.003	0.220	0.080	0.599
QR	2.37	1	0.124	0.462	0.172	1.236
Smoking × PON1 Q192R genotype	6.53	2	0.038	–	–	–
QQ × smoking	5.91	1	0.015	5.508	1.392	21.803
QR × smoking	1.24	1	0.265	2.161	0.558	8.377
Smoking	3.55	1	0.060	0.272	0.702	1.054
PON1 Q192R genotype	10.47	2	0.005	–	–	–
QQ	10.36	1	0.001	0.145	0.045	0.470
QR	4.68	1	0.030	0.277	0.087	0.886
Smoking × PON1 Q192R genotype	6.59	2	0.037	–	–	–
QQ × smoking	6.59	1	0.010	8.091	1.642	39.879
QR × smoking	3.56	1	0.059	4.673	0.942	23.193
Age	4.98	1	0.026	1.038	1.005	1.073
Gender (female)	1.61	1	0.205	1.481	0.807	2.718
Mood versus no mood disorders	0.046	1	0.829	0.939	0.533	1.657
Plasma PON1 activity	2.06	1	0.151	1.004	0.998	1.010
Serum HDL cholesterol	40.33	1	<0.001	0.906	0.879	0.934

smoking as explanatory variables showed that severe (Wald = 5.27, df = 1, $P = 0.022$), but not moderate (Wald = 2.61, df = 1, $P = 0.11$) nicotine dependence predicted the MetS. Entering smoking 10 or less, 11–20, 21–30, or 31 or more cigarettes per day versus non-smoking as explanatory variables showed that only 31 or more cigarettes per day significantly predicted the MetS (Wald = 13.59, df = 1, $P < 0.001$).

The first logistic regression analysis in Table 3 shows that PON1 Q192R genotype (QQ is inversely associated with MetS) and the smoking by PON1 Q192R interaction pattern, but not smoking *per se*, increased the odds of belonging to the MetS group ($\chi^2 = 16.1442$, df = 5, $P = 0.006$; Nagelkerke = 0.067; the number of correctly classified cases was 71.0%). The interaction showed that smoking by QQ carriers increased the odds to MetS.

The second logistic regression in Table 3 shows that the effects of PON1 Q192R genotypes (QQ and QR are inversely associated with MetS) and the interaction smoking by PON1 Q192R genotypes remained significant after considering the effects of HDL, age, and sex ($\chi^2 = 80.81$, df = 10, $P < 0.001$; Nagelkerke = 0.309; the number of correctly classified cases was 76.7%). Smoking *per se*, plasma PON1 activity, and mood disorders were not significant in this analysis. Entering unipolar depression and bipolar disorder versus no mood disorders as an explanatory variable (instead of mood versus no mood disorders) showed that these mood disorders had no significant impact (Wald = 3.42, df = 2, $P = 0.181$). Entering use of any medications as an additional explanatory variable showed that the use of medications was inversely associated with MetS (Wald = 5.38, df = 1, $P = 0.020$, $B = 0.494$, 95% lower and upper confidence

interval: 0.272 and 0.897, respectively) and that the effects of PON1 Q192R genotypes and the interaction PON1 Q192R genotype by smoking remained significant. Adjusting for other putative predictors showed that these were not significant and that the effects of PON1 genotype and the smoking by genotype interaction remained significant, i.e. ethnicity (Wald = 0.29, df = 1, $P = 0.587$); use of alcohol (Wald = 0.79, df = 1, $P = 0.375$); marital status (Wald = 1.95, df = 1, $P = 0.162$); years of education (Wald = 0.09, df = 1, $P = 0.770$), and use of statins (Wald = 0.26, df = 1, $P = 0.609$).

Interaction smoking by PON1 Q192R genotypes

In order to further examine the interaction between smoking and PON1 Q192R genotype, we performed additional logistic regression analyses in QQ, QR, and RR carriers and in smokers and non-smokers separately. We found that smoking was a significant explanatory variable increasing the odds of the MetS in QQ carriers (Wald = 9.91, df = 1, $P = 0.002$), but not in QR (Wald = 0.68, df = 1, $P = 0.409$) or RR carriers (Wald = 0.63, df = 1, $P = 0.428$). Table 4 shows the outcome of logistic regression analyses with the same variables as the second regression in Table 3 but performed in non-smokers and smokers separately. In non-smokers, we found that the PON1 genotypes QQ and QR and HDL cholesterol were inversely associated with MetS ($\chi^2 = 57.84$, df = 7, $P < 0.001$; Nagelkerke = 0.397; the number of correctly classified cases was 83.0%). In smokers, we found that HDL cholesterol was inversely associated with MetS, while age showed a positive relationship ($\chi^2 = 23.41$, df = 7, $P = 0.001$; Nagelkerke = 0.207; the number of correctly classified cases was 72.0%).

Table 4 Results of bivariate logistic regression analyses with MetS as dependent variable and the listed variables as explanatory variables and performed in non-smokers and smokers separately

	Wald	df	P	Odds ratio	95% Lower CI	95% Upper CI
Non-smokers						
PON1 Q192R genotype	10.84	2	0.004	–	–	–
QQ	10.79	1	0.001	0.114	0.031	0.416
QR	5.43	1	0.020	0.221	0.062	0.786
Age	0.63	1	0.429	1.021	0.970	1.075
Gender	2.24	1	0.134	0.488	0.191	1.248
Mood versus no mood disorders	0.68	1	0.408	0.685	0.279	1.679
Plasma PON1 activity	3.62	1	0.057	1.009	1.000	1.017
Serum HDL cholesterol	28.01	1	<0.001	0.871	0.827	0.917
Smokers						
PON1 Q192R genotype	0.063	2	0.969	–	–	–
QQ	0.045	1	0.833	1.120	0.392	3.202
QR	0.055	1	0.808	1.138	0.401	3.227
Age	4.64	1	0.030	1.049	1.004	1.096
Gender	0.32	1	0.573	0.793	0.353	1.779
Mood versus no mood disorders	0.12	1	0.730	1.143	0.534	2.446
Plasma PON1 activity	0.11	1	0.740	1.001	0.994	1.009
Serum HDL cholesterol	14.67	1	<0.001	0.929	0.895	0.965

Shown is the odds ratio *B* and the 95% upper and lower confidence interval (CI) values.

BMI, obesity, and overweight versus the MetS

Entering BMI as an additional explanatory variable in the full logistic regression depicted in Table 3 showed that BMI was significantly associated with MetS (Wald = 27.43, df = 1, $P < 0.001$, $B = 1.187$, 95% lower and upper confidence interval: 1.113 and 1.265, respectively) and that the effects of PON1 Q192R genotypes and the interaction PON1 genotype by smoking remained significant after adjusting for BMI. In order to delineate whether the PON1 Q192R genotypes may predict BMI, we carried out a multinomial regression analysis with obesity (BMI ≥ 30) and overweight (BMI between 25 and 30) as dependent variables (reference group: subjects with BMI < 25). Table 5 shows that two variables were significant in predicting obesity or overweight ($\chi^2 = 45.48$, df = 20, $P = 0.001$; Nagelkerke = 0.147), i.e. HDL cholesterol was significantly and inversely associated with overweight (Wald = 14.11, df = 1, $P < 0.001$, $B = 0.961$) and obesity (Wald = 9.53, df = 1, $P = 0.002$, $B = 0.961$) and male gender was

significantly and negatively associated with obesity (Wald = 5.89, df = 1, $P = 0.015$, $B = 0.400$), but not overweight (Wald = 0.98, df = 1, $P = 0.322$).

Discussion

The first major finding of this study is that PON1 Q192R functional genotypes were significantly associated with the MetS. We observed that the QQ and QR genotypes were protective and decreased the odds of having the MetS and thus that RR carriers are more likely to be diagnosed with the MetS. These findings are in accordance with a previous report that the RR allele is significantly associated with an increased risk of the MetS²⁶ and with the a priori hypothesis that the RR genotype may confer an increased risk of MetS. For example, a significant association between the R allele or the RR genotype and increased risk for CVD has been established by Liu *et al.*²⁴ PON1 RR/QR genotypes and especially the RR genotype are associated with vascular disease, such as stroke and myocardial infarction.⁴⁸ In hypertensive individuals, the 192 R allele augments the risk of stroke.⁴⁹ In another study, carriers of the RR (odds ratio 16.24 (6.41–41.14)), but also the QR (odds ratio 2.73 (1.57–4.72)) genotypes had a significant higher risk of coronary artery disease.⁵⁰ Also in an Iranian study, the PON1 R allele was found to be an independent risk factor for coronary artery disease.⁵¹ The PON1 R allele and RR genotype are increased in patients with coronary artery disease.⁵² In other studies, however, the PON1 Q allele was associated with significantly higher LDL and a worse outcome of CVD.⁵³ Individuals with the QQ genotype have a higher risk of all-cause mortality and cardiac events than RR and QR individuals.⁵⁴

Table 5 Multinomial regression analysis with obesity, i.e. BMI ≥ 30 and overweight, i.e. BMI between 25 and 30, as dependent variables and the listed variables as explanatory variables

Variables	χ^2	df	P
Smoking*	0.00	0	–
PON1 Q192R genotype*	0.00	0	–
Smoking $\times$ PON1 Q192R genotype	3.94	4	0.415
Age	3.073	2	0.215
Gender	6.21	2	0.045
Mood versus no mood disorders	2.11	2	0.349
Plasma PON1 activity	1.81	2	0.405
Serum HDL cholesterol	18.97	2	<0.001

*This reduced model is equivalent to the final model because omitting the effects does not increase the degrees of freedom.

Our findings that plasma PON1 activity is not lowered in individuals with MetS as compared to those without MetS is not in agreement with previous findings showing lowered PON1 activity in individuals with the MetS.^{18–23} However, these discrepancies may be explained by differences in PON1 assays. Q192R polymorphism is known to affect PON1 catalytic activity in a substrate-dependent manner.^{47,55} For example, R192 alloforms have a greater catalytic activity for hydrolysis of paraoxon and chlorpyrifos oxon than Q192 alloforms, Q192 alloforms show a higher activity against some nerve agents, and both alloforms show the same activity against PA and diazoxon.^{47,55} In our study, we measured PON1 total catalytic (hydrolysis) activity against PA, which at the low salt concentrations used here is not affected by PON1Q192R polymorphism.^{47,55} Previous studies in the MetS measured paraoxonase or arylesterase activity or PON1 concentrations using enzyme-linked immunosorbent assay methods.^{20–22} Moreover, PONs are primarily lactonases/lactonizing enzymes, with overlapping substrates and distinctive substrate specificities, although their natural substrates are not well characterized.⁵⁶ A recent report showed that – until more specific PON1 activity assays employing natural substrates are developed – results of clinical studies may differ depending on the assays that were employed.⁵⁷

We found that current smoking and especially severe nicotine dependence (according to scores on the Fagerström Nicotine Dependence Scale) and use of more than 31 cigarettes per day were significantly associated with the MetS. These findings are in accordance with previous studies showing a significant association between smoking and the MetS.^{27–29} Slagter *et al.*²⁹ additionally found a dose-dependent association between current tobacco use and the MetS. In our study, however, the relationship between tobacco consumption and the MetS was present for heavy tobacco use only, defined as more than 31 cigarettes per day.

The second major finding is that the abovementioned effects of smoking predicting the MetS could be attributed to the interaction between smoking and the PON1 genotypes. Thus, the effects of smoking increasing the odds of MetS were confined to QQ individuals and could not be detected in QR and RR individuals. These results corroborate those of a previous study showing that smoking was significantly associated with a higher risk for myocardial infarction only among QQ homozygotes.⁵⁸ In another study, smoking increased the risk of ischemic stroke in QQ homozygotes slightly more than in QR + RR individuals, i.e. odds ratio = 2.84 versus 2.33, respectively.⁴⁹ Such effects may be explained by pathophysiological factors, such as findings that the Q isoform confers

reduced protection against oxidation of LDL and HDL.²⁵ Thus, smoking could enhance lipid peroxidation especially in QQ carriers thereby increasing the odds of the MetS and thus CVD in those individuals. Interestingly, a significant interaction between smoking and PON1 genotypes on coronary heart disease risk was also established for the PON1 L55M polymorphism.⁵⁹ Interestingly, when MetS and diabetes are present PON1-192RR is associated with an increased risk of coronary stenosis especially in smokers.⁶⁰ All in all, the results show that MetS risk associated with smoking may be attributable to interactions with PON1 Q192R polymorphisms.

In this study, we found no significant association between mood disorders, including unipolar depression and bipolar disorder, and the MetS. A recent meta-analysis performed on 29 cross-sectional studies showed that depression and the MetS are significantly associated with a pooled estimate of 1.42 (95% confidence intervals of 1.28–1.57).³ These discrepancies may perhaps be explained by diagnostic issues, i.e. we used formal DSM-IV diagnosis of mood disorders, while Pan *et al.*³ showed that the association between depression and the MetS was stronger for self-rated depression (including also subsyndromal depression) than for diagnosis of major depression based on structural interviews. Nevertheless, cohort studies also showed that MetS significantly predicts future clinical depression and that depression may predict future MetS.³ In line with this, reciprocal relationships appear to exist between depression and CVD, depression and diabetes, and depression and obesity.^{61,62} The bidirectional relationships between mood disorders and the MetS may be explained by shared environmental risk as well as disorders in lipid metabolism, including lowered HDL cholesterol, and activation of immune-inflammatory and O&NS pathways (see the Introduction section).

Limitations of the study are the relatively small sample size of the study, the lack of a more complete PON1 genotyping, and the lack of a more complete evaluation of PON1 activity by means of assays on other substrates, including paraoxon and the newer substrate 5-thiobutyl butyrolactone. Another limitation of the study is that reports derived from ‘association studies constitute tentative knowledge and must be interpreted with caution’.⁶³

In conclusion, this study suggests that the MetS is associated with PON1 Q192R genotypes and a smoking by genotype interaction, but not with lowered PON1 total catalytic activity against PA. The QQ and QR genotypes appear to be protective while smoking by QQ genotype interactions increases the risk of MetS. Severe nicotine dependence and heavy smoking are significantly associated with the MetS, but these effects are no longer significant after

considering the effects of PON1 Q192R genotypes and smoking by genotype interactions. The effects of smoking increasing the risk of the MetS appear to be confined to QQ carriers.

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Conflicts of interest None.

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